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Tissue Geometry Regulates Collective Cell Motility
Sriram Manivannan, Jason P. Gleghorn, Jacquelyn Nestor, Celeste M. Nelson.
Princeton University, Princeton, NJ, USA.
Collective cell migration plays an important role in embryonic development, wound repair and cancer invasion. Certain cell types have an intrinsic ability to organize themselves and move collectively when they are confined within monolayers. Here, we explored the role of tissue geometry on the collective motility of epithelial cells. In particular, we used microlithography and time-lapse imaging to ask whether tissue geometry affects multicellular polarity and supracellular organization that are necessary for collective cell motion. We found that epithelial cells within monolayers tended to rotate as a group, and that increasing the size of the tissue increased the collectiveness of group rotation. The shape and boundary conditions of the tissue organized the motion of the cells by altering group rotation and coherence. The motility parameters of individual cells, including speed and persistence, were also affected by tissue geometry. Our results suggest that the overall architecture of the tissue in which cells reside instructs their movements with respect to each other within a collective. Accurate recapitulation of in vivo tissue structure will benefit future studies of processes which involve collective cell migration.

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Fibronectin Matrix Assembly Regulates Shear Stress-Induced Structural Remodeling and Motility
Rachel E. Evans, Brian P. Helmke.
University of Virginia, Charlottesville, VA, USA.
Complex spatial profiles of shear stress have been implicated in focal development of atherosclerotic lesions by mechanisms involving force transmission through endothelial cell (EC) focal adhesions to cytoskeleton. Fibronectin is a primary component of the provisional extracellular matrix (ECM) deposited in atherosclerotic lesions. Integrin-mediated mechanosignaling pathways in ECs are required for both adaptation to shear stress and fibronectin fibrillogenesis, but how fibronectin assembly state modulates EC responses to shear stress remains unknown. To investigate this question, focal adhesion displacement, cytoskeletal reorganization, and migration were measured before and after a step increase from 0 to 12 dyn/cm^2 steady unidirectional shear stress acting on ECs interacting with either assembled, fibrillar fibronectin matrix or unassembled, fragmented fibronectin on glass. Shear stress onset induced the arrest of focal adhesion displacement in ECs on unassembled fibronectin but not in cells on assembled fibronectin. In subconfluent layers, ECs on unassembled fibronectin migrated downstream after shear stress onset, but cells on assembled fibronectin migrated in random directions associated with local fibril orientations. In confluent monolayers, ECs interacting with unassembled fibronectin aligned in the flow direction faster than in monolayers on fibrillar fibronectin matrix, suggesting that fibronectin assembly regulated shear stress-induced cytoskeletal remodeling. Since cytoskeletal remodeling and focal adhesion displacement reflect early mechanosensing events after the onset of shear stress, these data suggest that fibronectin assembly regulates mechanosensitivity at the cell-matrix interface, which leads to shear stress-induced adaptation of cell motility and alignment.

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Human Mesenchymal Stem Cells Migration on Matrices with Distinct Elasticity Gradient Magnitudes
Ludovic G. Vincent, Yu Suk Choi, Adam J. Engler.
University of California San Diego, La Jolla, CA, USA.
Adult mesenchymal stem cells (MSCs) respond to extracellular niche elasticity, which varies dramatically between tissues that MSCs inhabit. Similarly, as MSCs egress from bone marrow and hone to tissues, they may encounter stiffness gradients brought on either by pathological conditions, e.g. myocardial infarction ~8.7 ± 1.5kPa/mm, or through normal tissue variation, e.g. muscle ~0.6 ± 0.9kPa/mm. We have recently shown that MSCs can undergo directed migration in response to shallow, physiological (~1kPa/mm) stiffness gradients before differentiating, suggesting the importance of spatial changes in stiffness. Such gradients, however, contain aphysical ranges, e.g. 1-15kPa, and more refined gradients of both range and gradient strength that mimic tissue interfaces are needed to better understand how mechanical cues dictate MSC migration versus differentiation. Using a polydimethylsiloxane microchannel mixer, we generated a polymer solution with a one-dimensional crosslinker concentration of constant monomer and photoinitiator but varying crosslinker. Photopolymerizing the solution inside the device yields a 3mm wide hydrogel with varying mechanical properties. Stiffness gradients of varying strength (1-30kPa/mm) and range (0.1-100 kPa) are achieved by varying the relative concentration of crosslinker from the input solutions. MSCs responded to stiffness gradients with a physiological range, e.g. mimicking the myotendenous junction, but of varying strength, i.e. 1-30kPa/mm. However, migration velocities of MSCs on gels of varying gradient strength were similar. Cell morphology was stiffness dependent with cells exhibiting increased spread areas on the stiffer regions, suggesting that the previously observed correlation between substrate mechanics, cell motility, and morphology exists over a physiological stiffness range but is independent of gradient strength. Efforts to define optimal myogenic stiffness and studies with C2C12 muscle cells are ongoing. These findings imply that MSCs in vivo may contribute better to repairs in stiffer regions of tissues where they may preferentially accumulate.

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Dynamic Surface Topography Influences Cell Function
Jennifer Kiang[1], Nathan Sniadecki[2], Adam J. Engler[1].
[1]University of California, San Diego, La Jolla, CA, USA, [2]University of Washington, Seattle, WA, USA.
Micro- and nano-scale changes in surface topography can modulate mesenchymal stem cell (MSC) differentiation; rough surfaces have been shown to induce osteogenesis to varying degrees depending on the scale and nature of the topographical features. While responses to static surfaces are novel, topography *in vivo* is constantly being remodeled by cells within the niche. To better understand how mesenchymal stem cells respond to changes in topography over time, we developed a soft polyacrylamide hydrogel with magnetic nickel microwires randomly oriented in the surface of the material. Varying the magnetic field around the microwires can reversibly induce their alignment with the direction of the field, causing the smooth hydrogel surface to develop small wrinkles. By varying the density of wires in the hydrogel, surface roughness changes, ΔR_{RMS}, can range from 0.05 to 0.62 μm as measured by traction force microscopy; this range encompasses roughness values for static surfaces. Time-dependent topographical changes are achieved by oscillating the field around the microwires, and ongoing efforts using step function and cyclic changes in topography indicate that both smooth muscle cells and MSCs change their behavior in response to dynamic surface topography, e.g. distinct changes in cytoskeletal structure as well as lineage marker expression is expected. Being able to dynamically study how cells respond to changes in topography will improve our current understanding of topography-driven effects on stem cell differentiation.

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Nanotopography Driven Mesenchymal Stem Cell Differentiation and Proliferation
Dustin R. Berger, Ketul Popat, **Ashok Prasad**.
Colorado State University, Fort Collins, CO, USA.
Plastic-adherent, fibroblast-like populations of bone marrow cells, termed multipotent mesenchymal stromal/stem cells (MSCs), constitute a tissue specific stem cell population that is responsible for tissue homeostasis and repair of bone, cartilage and adipose tissue. Previous work with MSCs had shown that they are capable of differentiation into specific lineages in response to the physical properties of the extracellular matrix such as matrix elasticity and cell geometry. Remarkably MSCs have been demonstrated to differentiate into bone-forming osteoblast cells by modifying the substrate nanotopography. In this work, the role of nanotopography on the long-term response of MSCs is explored using biodegradeable polycaprolactone (PCL) nanopillar and nanofiber surfaces seeded with rat MSCs and cultured in normal growth media for four weeks. We found that after four weeks in culture under normal expansion media conditions, MSCs cultured on nanofibers exhibit better adherence, significantly increased proliferation, and maintain increasingly dense fibroblast-like morphologies. In contrast, MSCs seeded on nanopillar surfaces display lowered adherence, reduced proliferation, and adopt highly elongated cellular morphologies. Cell shape and area quantification reveals that MSCs cultured on smooth substrates adopt uniformly spread morphologies covering large surface areas, while MSCs cultured on nanopillars have significantly larger length-to-width ratios and significantly reduced coverage areas. Immunofluorescent staining of MSCs on PCL nanopillars reveals the presence of two bone marker proteins, osteopontin and osteocalcin, providing evidence for differentiation into osteoblast-like cells. Unlike the nanopillar topography, MSCs cultured on nanofiber and smooth PCL surfaces did not appear to